Florida Citrus Advanced Technology Program

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Psyllid	10/15/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Efficient acquisition of Candidatus Liberibacter asiaticus by nymphs of Asian citrus psyllid

TITLE and CONTACT INFORMATION

Proposal Title Factors influencing acquisition and inoculation of Candidatus Liberibacter asiaticus by Diaphorina citri

Principal Investigator Joao Roberto Spotti Lopes	*PI Last Name* Lopes
Email jlopes@esalq.usp.br	*FDACS Contract Number* NAS ID Lopes-126
Phone +55 (19) 3429-4199, ext. 212	*Project Duration (years)* 3 *Year of Project* 1
Organization University of Sao Paulo	*Total Direct Funds (current year)* $63,200.00

REPORT UPDATE (650 words; provide details about your headline)

This project's goal is to determine factors influencing transmission efficiency of Candidatus Liberibacter asiaticus by the Asian citrus psyllid (ACP), Diaphorina citri, which have implications on current HLB management strategies, particularly rouging and vector control. We are currently studying how acquisition efficiency varies depending on vector developmental stage (study 1), citrus phenology (young leaves x mature leaves) (study 2) and pathogen titer and symptom expression in source plants (study 3). We will also study the time period required for psyllid adults to inoculate the pathogen in healthy citrus (study 4) and if a systemic insecticide can affect this process (study 5). So far we have started experiments related to studies 1-4, and have partial results for studies 1 and 2, which are described below. The project is progressing as planned in the original proposal.

In study 1, we are comparing different ACP nymphal stadia (1st, 2nd, 3rd, 4th and 5th instars) and adults (1 wk old) with respect to acquisition efficiency of Ca. L. Liberibacter on citrus. Around 10-20 psyllid adults and nymphs of each instar were confined on distinct leaves of a young shoot of a symptomatic infected plant, with recently expanded leaves, inside leaf cages. After an acquisition access period (AAP) of 48 h, the insects of each age treatment were first transferred to healthy citrus seedlings for a latent period of 15 days, and then transferred to healthy test seedlings (5 insects/plant) for a 7-day inoculation access period (IAP). After the IAP, total DNA of each insect was extracted and the sample was submitted to real-time PCR. In a first trial of this experiment (set up in May/09), the results suggested that nymphs acquire the bacterium more efficiently than the adults. Two recent trials set up in July/09 and Sept/09 confirmed this trend; mean psyllid infectivity rates of 100, 79, 80, 75, 83 and 60% were obtained when acquisition occurred during the 1st, 2nd, 3rd, 4th, 5th instars and adults, respectively. We are still waiting the data of a fourth trial (Oct/09) to run a statistical analysis, but these partial results indicate that the pathogen is efficiently acquired by all nymphal instars of ACP.

In study 2, we observed the effect of leaf age (young and asymptomatic x symptomatic mature leaves) in source plants infected with Ca. L. asiaticus on acquisition efficiency and probing behavior of ACP adults. In a first experiment, groups of healthy adults were confined separately on a fully-expanded young leaf and on a mature (symptomatic) leaf of source plants of Ca. L. asiaticus. After a 4-day AAP, psyllids from each treatment were kept on healthy plants for 24-day latent period and then tested for infectivity by qPCR. ACP adults acquired Ca. L. asiaticus with higher efficiency on young (asymptomatic) (20.4% infective individuals) than on mature (0%) leaves from infected plants. We now repeated this experiment with inclusion of a third leaf age treatment (not fully-expanded young leaves), and observed that acquisition efficiency on mature leaves was lower (6.0% infective individuals) than on fully-expanded (25.1%) or not fully-expanded (9.1%) young leaves, as reported previously. By analyzing the probing behavior of adult females of ACP on mature x young (fully-expanded) leaves of infected citrus by the Electrical Penetration Graph (EPG) technique, we noted that phloem ingestion was longer and observed more often on young leaves. Within 5 h, around 50% individuals on young leaves started sustained phloem ingestion (E2), whereas less than 15% individuals on mature leaves did so. On mature leaves, the insects spent most of the time with the stylets in the parenchyma (pathway phase) or non-probing. The higher frequency and longer duration of phloem ingestion appears to explain at least in part the higher acquisition efficiency of Ca. L. asiaticus when ACP is confined on young asymptomatic leaves.